Sussex Co.: swamp west of Springdale at Big Spring near Newton, *Dowell 4929* (NY, US), *Dowell 5033* (NY); Sussex Co.: roadside 1 mi south of Greendell, *Edwards s. n.* (NY); Bearfort Mtn., *W. D. Miller 1648* (NY); West Englewood, *Carhart 2b* (NY) 2 sheets. **New York:** Green Lake, Jamesville, *W. R. Dunlop s. n.* (NY); Harris Swamp near Pilot Knob, *Benedict s. n.* (US-2202218); Kirkville, *L. M. Underwood s. n.* (NY); Staten Island: Arlington Station, *Dowell 2801a & b* (US). **Ohio:** Geauga Co.: *Hopkins s. n.* (NY). **Pennsylvania:** Berks Co.: swamp along spring-run 1.5 mi ne of Bernharts, *Wherry s. n.* (US-1849217); Delaware Co.: valley of Cruise Creek, *Poyser 1286* (NY); Wakefield, Lanbor, *J. J. Carter s. n.* (NY). **Vermont:** N. Lynnfret (Limfret?), *A. P. & L. V. Morgan s. n.* (US-154517).

In the garden, its vegetative propagation should be promoted and supplemented with modern tissue culture techniques to meet horticultural demands. It is a large and vigorous fern that forms extensive colonies through vegetative expansion while under cultivation. One specimen from New York was planted at the New York Botanical Garden, Bronx, NY in 1960 by members of the American Fern Society. Thirty-five years later, that plant had formed a clone 4 m across with over 200 apices (Mickel, 1994). JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

Cryopreservation of Shoot Tips of *Selaginella uncinata*.—Cryopreservation, or storage in liquid nitrogen (LN), has been successful for a wide variety of plant tissues, including shoot tips of *in vitro* cultures of higher plants (Bajaj, *In* Y. P. S. Bajaj, *Cryopreservation of Plant Germplasm I, Biotechnology in Agriculture and Forestry* 32. Springer, Berlin, 1995). At the temperature of LN, −196°C, long-term, stable storage of rare, endangered, or other valuable plant germplasm can be achieved. In an attempt to extend this technology to pteridophytes, LN storage of shoot tips of *in vitro* grown *Selaginella uncinata* was tested using the encapsulation dehydration procedure (Fabre and Dereuddre, Cryo-Letters 11:413–426, 1990).

Shoot cultures of *Selaginella uncinata* (Desv. ex Poir.) Spring. were established from a plant purchased from Carolina Biological Supply Co. (voucher specimens deposited at the University of Cincinnati Herbarium, CINN, and at the CREW Herbarium). Tissues were surface disinfested in a 1:20 dilution of commercial sodium hypochlorite for five minutes, followed by two rinses in sterile, ultrapure water. The tissues were then transferred to a basal medium consisting of half-strength Murashige and Skoog salts with minimal organics (MS medium; Linsmaier and Skoog, Physiol. Plant. 18:100–127, 1965) with 1.5% sucrose and 0.22% Phytagel (Sigma Chemical Co.). Cultures were maintained on this medium in 60 × 15 mm plastic petri plates, approximately 15 ml of medium/plate, at 26°C under Cool White fluorescent lights with a 16:8 hr, light:dark cycle. For preculture, one week prior to freezing the tissues were transferred to fresh basal medium or to basal medium plus 10 μM abscisic acid (ABA), which was filter sterilized and added to the medium after autoclaving.

TABLE 1. Survival of shoot tips of *S. uncinata* through the steps of the encapsulation dehydration technique, 3–15 clean shoot tips per treatment. Encapsulated shoot tips were pretreated in 0.75 M sucrose, dried and exposed to LN, and samples were cultured after each step. Results are the mean of three experiments (±SE), except for long term LN exposure, for which there was one trial. Numbers of samples per trial are given in parentheses.

	Percent survival[1]			
Preculture medium	Pretreated	Dried	1 hr LN exposure	3.5 yr LN exposure
Basal	79 ± 7[a] (12, 13, 13)	25 ± 6[bc] (12, 13, 10)	11 ± 7[b] (8, 15, 11)	0 (14)
Basal + ABA	90 ± 6[a] (12, 9, 5)	92 ± 8[a] (12, 7, 5)	57 ± 18[ac] (3, 9, 6)	44 (11)

[1] Values with different superscripts are significantly different to the 0.05 level.

For cryopreservation, the procedure of Fabre and Dereuddre (1990) was followed. Shoot tips approximately 2–3 mm in length were excised and placed in a solution of 3% alginic acid with 0.75 M sucrose in calcium-free MS medium. The solution containing the tips was then added dropwise to a solution of 100 mM $CaCl_2$ to form the alginate beads. These were incubated for 18 hr in liquid MS medium containing 0.75 M sucrose and were then dried for 4 hours in open dishes on filter paper under the air flow of the laminar flow hood. The dried beads containing the shoot tips were then transferred to 2 ml polypropylene cryovials and immersed directly into LN. After 1 hr, the cryovials were removed from the LN and allowed to warm at room temperature for 20 minutes. The encapsulated shoot tips were then transferred to basal medium for rehydration and recovery growth. Survival was measured as regrowth from a shoot tip. Results were analyzed by ANOVA (Tukey HSD Test, StatSoft, QuickSTATISTICA software).

Drying and LN exposure significantly decreased survival from shoot tips which were precultured on basal medium alone (Table 1). When shoots were precultured for one week on basal medium plus 10 μM ABA, there was no decrease in survival when the tissues were dried, although survival did decrease to approximately half when the tissues were exposed to LN. However, survival of both dried and LN exposed tissues was significantly better than that of control tissues.

Shoot tips which were prepared using the encapsulation dehydration procedure with and without ABA preculture were also transferred to LN for long-term storage. When these were removed after 3 1/2 yrs of storage in LN and recultured on basal medium, there was no regrowth from shoot tips which had been isolated from tissues grown on basal medium. However, 44% of the shoot tips which had been precultured on basal medium plus ABA initiated good growth after eight weeks (Figure 1).

These results demonstrate that shoot tips of *S. uncinata* can survive LN exposure and long-term storage in LN when they are prepared using the encapsulation dehydration procedure in combination with preculture on ABA.

FIGURE 1. Shoot tip of *S. uncinata* growing out of alginate bead, 8 weeks after exposure to LN. 6.7 X

Encapsulation dehydration is a technique which has been successful with shoot tips of a number of species of angiosperms (Touchell and Dixon, *In* B. G. Bowes. *Atlas of Plant Propagation and Conservation.*, Manson Publishers, London, 1999) as well as with gametophytes of ferns and bryophytes (Pence, The Bryologist 101:278–281, 1998 and Amer. Fern J. 90:16–23, 2000), and it is not surprising that it can also be successful with shoot tips of the fern allies as well.

Survival of *S. uncinata* through cryopreservation was low unless the tissue was precultured on ABA. Treatment with ABA has been shown to initiate desiccation tolerance in the fern *Polypodium virginianum* L. (Reynolds and Bewley, J. Exper. Bot. 44:921–928. 1993a and J. Exper. Bot. 44:1771–1779, 1993b), as well as in bryophytes (Hellwege et al., Planta 198:423–432, 1996; Pence, 1998), and ABA is involved in several aspects of desiccation tolerance and water stress in seed plants (Hartung and Davies, *In* W. J. Davies and H. G. Jones, Abscisic Acid. Physiology and Biochemistry. BIOS Scientific Publishers, Oxford, 1991). It appears that preculture on ABA improves tolerance of *S. uncinata* shoot tips to the stresses of drying and LN exposure in the encapsulation dehydration procedure, perhaps mimicking a natural response to stress in this species. Several other species of *Selaginella* are known to be "resurrection plants," with a remarkable ability to tolerate extreme water loss

(see Proctor and Pence, *In* M. Black and H. Pritchard. *Desiccation and Plant Survival.* CAB International, Oxon, U.K., in press).

Although encapsulation dehydration was successful in preserving shoot tips of *S. uncinata,* preliminary studies in this laboratory indicate that the survival of some other species of *Selaginella* through this procedure may not be as high (unpublished results). Species differ in their ability to survive LN exposure, and other techniques, such as slow freezing or vitrification, may be more successful with other species in this genus (Fukai et al., Euphytica 56:149–153, 1991; Yamada et al., Plant Science 78:81–87, 1991).

There are over 700 species of pteridophytes which are of conservation concern worldwide, including 23 species of *Selaginella* (Walter and Gillett, 1997 IUCN Red List of Threatened Plants. IUCN—The World Conservation Union, Gland, Switzerland 862 Pp., 1998). Further studies should reveal how broadly applicable the encapsulation dehydration procedure is to shoot tips of pteridophytes and how cryopreservation may be used for preserving *Selaginella* germplasm for research and conservation.—VALERIE C. PENCE, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220.